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YEAST EXTRACT COMPOSITION, METHOD FOR MANUFACTURING THE SAME, AND FODDER INCLUSIVE OF THE SAME

[Koso Ekisu Soseibutsu Oyobi Sono Seizo Ho Narabi ni Sore o Ganyu Suru Shiryo]

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(54) Title of the invention

YEAST EXTRACT COMPOSITION, METHOD FOR MANUFACTURING THE SAME AND FODDER INCLUSIVE OF THE SAME

(57) Summary

Objective: To develop a yeast extract composition endowed with both a fodder intake acceleration function and an immunopotentiation function absent among yeast extracts known in the prior art.

Constitution: A yeast extract composition endowed with a fodder intake acceleration function and an immunopotentiation function inclusive, as effective components, of 5'-nucleotide, free amino acid, β-glucan, and mannan, a method for manufacturing the same, and a fodder inclusive of the same.

Effects: The present composition can be used extensively as fodder additives for the purpose of preventing various infectious diseases.

[1] Numbers in the margin indicate pagination in the foreign text.

Patent Claims /2

Claim 1

A yeast extract composition endowed with a fodder intake acceleration function and an immunopotentiation function characterized by the inclusion of 5'-nucleotide, free amino acid, β-glucan, and mannan.

Claim 2

A yeast extract composition specified in Claim 1 which includes 1 ~ 5% each of 5'-inosinic acid & 5'-guanylic acid with respect to the solid content thereof, 12% ~ 40% of a free amino acid & 1% ~ 25% of β-glucan with respect to the solid content thereof, and, additionally, 1% ~ 25% of mannan.

Claim 3

A method for manufacturing a yeast extract composition characterized by the facts that intrabacterial enzymes are entirely deactivated by means of autodigestion & then heating, that cellular wall-soluble enzymes are subsequently exerted on the deactivated matter, and that 5'-inosinic acid & 5'-guanylic acid are subsequently exerted on the same for the purpose of

simultaneously elevating the 5'-nucleotide internalization ratio, free amino acid internalization ratio, and β-glucan & mannan internalization ratios.

Claim 4

A method for manufacturing a yeast extract composition specified in Claim 3 wherein a temperature of 45 ~ 65°C and a pH of 5.5 ~ 8.5 are designated as autodigestion conditions.

Claim 5

A method for manufacturing a yeast extract composition specified in Claim 3 or 4 wherein the temperature at the time of heating is 80 ~ 120°C.

Claim 6

A method for manufacturing a yeast extract composition specified in any one of Claim 3 ~ 5 wherein said yeast is a Torula yeast or Saccharo yeast.

Claim 7

A method for manufacturing a yeast extract composition specified in any one of Claims 3 ~ 6 wherein said yeast is a yeast incubated in a sulfurous acid pulp effluent.

Claim 8

A fodder inclusive of the yeast extract composition specified in either of Claims 1 ~ 2.

Claim 9

A fodder inclusive of 0.1 ~ 20 wt% of the yeast extract composition specified in either of Claims 1 ~ 2.

Detailed explanation of the invention

[0001]

(Industrial application fields)

The present invention concerns a yeast extract composition, a method for manufacturing the same, and a fodder independent of the same, and more specifically, it concerns a yeast extract

composition endowed with both a fodder intake acceleration function and an immunopotentiation function, a method for manufacturing the same, and a fodder independent of the same.

[0002]

(Prior art and its problems)

Generally speaking, immune functions of fishes, domesticated animals, and domesticated fowls at their respective juvenile stages are insufficient, and accordingly, infectious diseases of their digestive tract system & respiratory system are likely to occur. It is therefore necessary to proliferate their growths for the purpose of bestowing them with resistances. Once these infectious diseases break out, furthermore, they are likely to spread easily in that fishes, domesticated animals, and domesticated fowls are generally bred at high densities from the standpoint of improving productivities, due to which extremely grave economic losses are experienced, and this problem has become an especially critical one in the field of industrial fish farm breeding. At present, various chemicals, led by antibiotics, are being used for preventing and/or treating these infectious diseases. Not only are the effects of these chemicals, however, insufficient, but secondary problems such as *in vivo* chemical accumulation, emergence of chemical-resistant germs, etc. also arise, and therefore, the uses of these chemicals are becoming increasingly restricted.

[0003]

As far as alternative methods are concerned, a method wherein a substance endowed with a fodder intake acceleration function is administered for purposes of accelerating growths and of abbreviating the duration of juvenile periods, which are prone to infectious diseases, a method wherein an immunopotentiation substance is administered for the purpose of inducing the acquisition of resistance against infectious diseases, etc. are being investigated. Known substances endowed with fodder intake acceleration functions are instantiated by 5'-nucleotide, free amino acid, peptide, sugar, etc. A method wherein a substance inclusive of these substances as effective components is added to a fodder for purposes of improving the appetite and of accelerating the

fodder intake is known (Japanese Patent Application Publication Kokai No. Hei 3[1991]-266944). There exists, however, a limit to the extent that the duration of juvenile periods prone to infectious diseases can be abbreviated, and thus, it cannot be said to be a fully satisfactory method by itself.

[0004]

As immunopotentiation substances, furthermore, β-glucan & mannan, etc., which are cellular wall constituent components of mushrooms, yeast germs, etc., are known. As immunopotentiation substances derived from mushrooms, Letinan, which is obtained by extracting Shiitake mushrooms with hot water, Schizophyllan, which is produced by *Schizophyllum commune Fr.*, etc. have been developed and marketed. As immunopotentiation substances that use yeast germs, furthermore, Zymozan, which includes β-glucan, namely a constituent component of the cellular walls of yeast germs, is known. The costs of these immunopotentiation agents, however, are high not only because cumbersome production processes are involved but also because their yields are also insufficient, and thus, they are problematic from the standpoint of extensively using them as fodder additives. Substances endowed with both immunopotentiation functions and body weight proliferation functions, furthermore, are also known (Japanese Patent Application Publication Kokai No. Hei 2[1990]-11519), although they are effective only in cases where their intakes are forcibly induced till achievements of glutting limits, and their effects, too, are not fully satisfactory. For the reasons cited above, the development of a substance endowed with both a fodder intake acceleration function and an immunopotentiation function has long been earnestly desired.

[0005]

(Mechanism for solving the problems)

The present inventors compiled exhaustive research in order to solve the aforementioned problems, as a result of which the present invention has been completed after it had been discovered that a yeast extract composition endowed simultaneously with both an extremely favorable fodder

intake acceleration function and an immunopotentiation function can be obtained in a case where autodigestion is induced within specifiably limited temperature & pH ranges for boosting the solid content yield and for abundantly enriching the free amino acid internalization ratio without entailing the decompositions of high-molecular-weight RNA & mannan, where the obtained reaction solution is heated under certain conditions for deactivating intrabacterial enzymes, where a cellular wall-soluble enzyme is then exerted onto the deactivated solution, and where 5'-phosphodiesterase & 5'-adenylic acid deaminase capable of generating 5'-nucleotide are subsequently added to the same.

[0006]

The temperature and pH of the autodigestion of the present invention serve as extremely important factors from standpoints of boosting the free amino acid internalization ratio and of inhibiting the decompositions of RNA & mannan, whereas the orchestration of a heating process after the autodigestion reaction is important from the standpoint of inhibiting the decompositions of taste-imparting 5'-nucleotide and β-glucan & mannan in the course of the subsequent enzymatic reaction process. In the following, the present invention will be explained in further detail. There are no special restrictions on the yeasts used in the present invention so long as they are selected from among edible and fodder-compatible ones, and as such, ones being used generally in the food industry such as beer yeasts, bread yeasts, alcohol yeasts, sake yeasts, etc. can be used. /3

[0007]

These yeasts are instantiated by *Saccharomyces cerevisiae* (IFO 1954, IFO 0309, & IAM 4274), *Candida utilis* (IFO 0619 & ATCC 15239), *Torulopsis nodaensis* (IFO 1942), *Torulopsis stellata* (IFO 1953), *Hansenula anomala* (IFO 1150), etc., and of these, Torula yeasts are especially desirable in that their RNA internalization ratios are high and that they are endowed with potent taste imparting potentials. Live germs obtained by washing incubation products are used as yeast germs, and in particular, yeasts incubated in sulfurous acid pulp effluents are excellent as the yeast of the present invention in that they are inexpensive and that their activities are high.

[0008]

After the yeast has been suspended at an appropriate concentration of approximately 10 ~ 15%, its autodigestion is induced. As far as the reaction pH and reaction temperature are concerned, conditions capable of inhibiting the decompositions of high-molecular-weight RNA & mannan and of boosting the generation of free amino acid are necessary, and the sought objectives can be achieved by confining the pH and temperature to ranges of 5.5 ~ 8.5 and 45 ~ 65°C, respectively. In a case where the pH deviates from this range, it becomes difficult to boost the free amino acid internalization ratio. In a case where the temperature is lower than this range, the free amino acid internalization ratio may become elevated, but the NRA and mannan become decomposed. In a case where the same exceeds 65°C, the decompositions of the NRA and mannan can be eradicated, but the free amino acid internalization ratio drops to an extremely low level. In a case where the free amino acid internalization ratio is lower than 12%, no notable fodder intake acceleration effect can be acknowledged. In a case where the internalization ratio exceeds 40%, on the other hand, the autodigestion time becomes significantly prolonged, due to which such problems as degradation, etc. arise. For these reasons, it is desirable for the free amino acid internalization ratio to be confined to the range of 12% ~ 40%.

[0009]

After the autodigestion has been executed under the aforementioned conditions for 10 ~ 20 hours or so, intrabacterial enzymes are deactivated by means of heating at 80 ~ 120°C, preferably 90 ~ 100°C. A duration of approximately 10 min. suffices as the heating time. Next, approximately 0.3 ~ 3% of a cellular wall-soluble enzyme is added, and a reaction is then induced for 1 ~ 5 hours. So long as the duration is confined to this range, no significant depolymerization of polysaccharide is incurred even if the cellular wall-soluble enzyme is added to the yeast obtained upon the completion of the previous process, based on which it becomes possible to elevate the solid content yield. It is assumed that the glucan & mannan within the cellular wall form a composite with protein, that the cellular wall becomes rigidified due to the modification of the protein as a result of

the heating treatment, and that the depolymerization accordingly becomes hindered. In a case where the reaction time is excessively brief, the β-glucan & mannan internalization ratios become lower than 1%, due to which not only does the immune activity become insufficient, but the solid content yield also decreases. In a case where the duration is excessively long, the β-glucan & mannan internalization ratios become as high as 25% or above, but since depolymerization progresses more than necessary, the immune activity inevitably diminishes. For these reasons, it is desirable for the internalization ratios of both β-glucan and mannan to be confined to 1 ~ 25%.

[0010]

There are no special restrictions on concomitantly used cellular wall-soluble enzymatic agents so long as they include glucanase and mannase and as their activities are sufficient for solubilizing the yeast cellular wall, whereas commercially sold cellular wall-soluble enzymes are instantiated by YL-5 (manufactured by Amano Pharmaceutical Co.), Tunicase (manufactured by Yamato Kasei Co.[]), Kitalase (Kumiai Chemical Co.), etc. Subsequently, 5'-phosphodiesterase and 5'-adenylic acid deaminase are added, as a result of which 5'-nucleotides become generated. There are no special restrictions on the enzyme addition ratio, enzyme reaction temperature, and pH, and conditions optimal for the respective enzymes may be adventitiously selected. After the reaction has been completed, the obtained reaction solution is heated at 90°C for deactivating the enzyme, and subsequently, the supernatant is centrifugally separated, enriched, recovered as an extract component, and then dried by an appropriate mechanism (e.g., spray dry, etc.). The yeast extract thus obtained is endowed with an excellent fodder intake accelerating effect in that it includes 1 ~ 5% each of 5'-inosinic acid & 5'-guanylic acid with respect to the solid content thereof as well as 12% ~ 40% of free amino acids. It is also endowed with an excellent immunopotentiation activity in that it includes 1 ~ 25% of β-glucan and 1 ~ 25% of mannan. Since its solid content yield is at least 50%, furthermore, it is extremely advantageous from an economic point of view, and it can therefore be utilized extensively as fodder additives. The optimal additive [sic: Presumably "addition ratio"] of the product of the present invention with respect to a fodder differs depending

on the types of targeted animals and their ages in weeks, although the objective sought by the present invention can be achieved so long as the addition ratio is confined to a range of 0.1 ~ 20 wt%, preferably 0.2 ~ 5%.

[0011]

(Application examples)

In the following, concrete application examples will be shown, although the present invention is in no way limited to them.

[0012]

Measurement of fodder intake acceleration activity

Test Example 1

After *Saccharomyces cerevisiae* (IFO 1954) had been incubated within a 5% molasses medium, germs were collected and then washed, as a result of which 1,000 mL of a yeast slurry (germ concentration: 15%) was prepared. After its pH had been adjusted at 6, it was reacted at 55°C over an 18-hour period. Upon the completion of the reaction, 1.5 g of a cellular wall-soluble enzyme (YL-5, trademark of & manufactured by Amano Pharmaceutical Co.) was added, and the contents were then reacted at 55°C over a 3-hour period. Next, the temperature of the obtained product was elevated to 70°C, and after 0.3 g of 5'-phosphodiesterase (Nuclease "Amano," trademark of & manufactured by Amano Pharmaceutical Co.) was added, and after the pH of the obtained mixture had been adjusted at 5, it was reacted over a 10-hour period. After 0.2 g of 5'-adenylic acid deaminase (Deamizyme, trademark of & manufactured by Amano Pharmaceutical Co.) had subsequently been added, the pH was adjusted at 5, and the obtained mixture was reacted over a 10-hour period. The product obtained upon the completion of the reaction was treated based on an ordinary method, as a result of which 122 g of a yeast extract was obtained. The 5'-inosinic

acid, 5'-guanylic acid, and free amino acid internalization ratios of the obtained yeast extract were quantified by means of high-speed liquid chromatography, as a result of which the respective values were 2.5%, 2.6%, and 45%, whereas the solid content yield was 81.3%. Moreover, the β-glucan and mannan internalization ratios were quantified by means of high-speed liquid chromatography in terms of differentials before & after hydrolysis, as a result of which the respective values were 9% and 8%. Moreover, their molecular weights were calculated based on the gel filtration method, as a result of which the respective values were 63,000 and 59,000.

[0013]

Test Example 2

After a Torula yeast had been incubated by using a 3% sulfurous acid pulp effluent /4 medium, germs were collected and then washed, as a result of which 1,000 mL of a yeast slurry (germ concentration: 15%) was prepared. After its pH had been adjusted at 6.5, it was subjected to an autodigestion reaction at 60°C over an 18-hour period. It was subsequently heated at 95°C over a 10-min. period for deactivating the intrabacterial enzyme, and after 1.8 g of a cellular wall-soluble enzyme (Tunicase, trademark of & manufactured by Yamato Kasei Co.) had then been added, the obtained mixture was reacted at 55°C over a 2.5-hour period. The product obtained upon the completion of the reaction was heated at an eventual temperature of 70°C, and after 180 mg of a nucleic acid decomposition enzyme (Nuclease "Amano," trademark of & manufactured by Amano Pharmaceutical Co.) had then been added, the obtained mixture was reacted over a 9-hour period. After its temperature had subsequently been lowered to 45°C, 1.8 g of protease (Amano P, trademark of & manufactured by Amano Pharmaceutical Co.) and 200 mg of 5'-adenylic acid deaminase (Deamizyme, trademark of & manufactured by Amano Pharmaceutical Co.) were added, and the obtained mixture was reacted over a 10-hour period. After the obtained product had been cooled, it was treated based on an ordinary method, as a result of which 105 g of a yeast extract was obtained. The 5'-inosinic acid, 5'-guanylic acid, and free amino acid internalization ratios of the

obtained yeast extract were quantified by means of high-speed liquid chromatography, as a result of which the respective values were 3.6%, 3.8%, and 35%, whereas the solid content yield was 70.0%. Moreover, the β-glucan and mannan internalization ratios were quantified by means of high-speed liquid chromatography, as a result of which the respective values were 12% and 20%. Moreover, their molecular weights were calculated based on the gel filtration method, as a result of which the respective values were 72,000 and 56,000.

[0014]

Reference

Schizophyllan:	MW:	10,000 ~ 80,000
Leticinan:		400,000
Pahimalan:		180,000
Glucan within Zymozan:		6,500

Mannan within yeast decomposition [product?]: 59,000

Estimated mannan internalization ratio of another company's yeast extract: 3% or less

Estimated glucan internalization ratio of another company's yeast extract: 1% or less

Mannan internalization ratio within HU: 12% (MW: 72,000)

Fodder intake acceleration functions & effects of four samples, namely the obtained two types of yeast extracts, a yeast extract derived from a commercial beer yeast, and a commercial grape juice added as a fodder intake accelerator at the suckling stage, were investigated.

[0015]

Application Example 1

A piglet fodder was prepared by adding 0.2% of each of the various samples to the base fodder shown in Table I.

[0016]

[Table 1]

Table I: Base fodder composition of artificial piglet milk formula

[Component]	[Mixing ratio (wt%)]
Wheat flour:	35
Defatted powdery milk:	35.5
Soybean protein:	10
Fish powder:	4
Grape sugar:	3
Oils & fats:	3
Vitamins & minerals:	2
Emulsifier:	0.5

[0017]

Ten weaned piglet littermates (2 weeks old) were each housed in a breeding cage, and after fodders to which the control and the respective samples had respectively been added had been fed to them over a 14-day period based on the selection method, their fodder intake levels were compared. Incidentally, the placement sites of the provided breeding boxes were swapped at a 1-day interval. The obtained results are shown in Table II {fodder intake ratio: Equivalent value of a case where the control artificial milk formula intake level is defined as 100 (same below)}.

[0018]

[Table 2]

Table II: Fodder intake ratios of the respective samples

S ample name	Control	Sample 1 of the present invention	Sample 2 of the present invention	Commercial yeast extract	Grape juice
Fodder intake ratio	100	117	119	101	103

[0019]

Application Example 2

A calf fodder was prepared by adding 0.2% of each of the various samples to a commercial calf artificial milk formula provided as a base fodder, and subsequently, six members per group of 1-week-old calves weaned from their mothers were evaluated according to procedures similar to those in Application Example 1. The obtained results are shown in Table III.

[0020]

[Table 3]

Table III: Fodder intake ratios of the respective samples

S ample name	Control	Sample 1 of the present invention	Sample 2 of the present invention	Commercial yeast extract	Grape juice
Fodder intake ratio	100	116	120	101	103

[0021]

Application Example 3

A chick fodder was prepared by adding 0.2% of each of the various samples to a commercial chick fodder provided as a base fodder, and subsequently, ten members per group of 1-week-old chicks were evaluated according to procedures similar to those in Application Example 1. The obtained results are shown in Table IV.

[0022]

[Table 4]

Table IV: Fodder intake ratios of the respective samples /5

S ample name	Control	Sample 1 of the present invention	Sample 2 of the present invention	Commercial yeast extract	Grape juice
Fodder intake ratio	100	115	118	102	104

[0023]

Measurement of immunopotentiation activity

The fodders prepared in Test Examples 1 & 2 were subjected to the following tests.

[0024]

Application Example 1: anti-complement activity test

After each sample had been added to a complement solution comprising of a properly diluted guinea pig blood serum, the obtained mixture was warmed at 37°C over a 30-min. period. Next, an antibody-sensitized sheep hemoglobin was added, and after he obtained mixture had been warmed at 37°C over a 60-min. period, the degree of hemolysis of the sheep hemoglobin was measured for determining the residual complement content which had not become activated by the sample and for gauging the complement secondary path activation function of the sample. Incidentally, a yeast extract derived from a commercial beer yeast, Zymozan (trademark of a yeast cellular wall component manufactured by Sigma Co.), and a commercial immunopotentiation agent

(Inorich 50M, trademark of & manufactured by Eizai Co.) were concomitantly subjected to anti-complement activity tests for comparative purposes. The obtained results are shown in Figure 1.

[0025]

Application Example 2: Microphage activation test

After each sample had been added to cells effused within a mouse abdominal cavity & incubated in a thioglycolate medium, the glucose content within the incubation supernatant was quantified 24 hours later, and the microphage activation function was gauged in terms of its consumption level. Incidentally, a yeast extract derived from a commercial beer yeast and a commercial immunopotentiation agent (Inorich 50M, trademark of & manufactured by Eizai Co.) were concomitantly subjected to microphage activity tests for comparative purposes. The obtained results are shown in Figure 2.

[0026]

Application Example 3: Carbon clearance test

Carbon particles diluted by 25 times (Rotring ink was used for convenience's sake) were dispensed into tail veins of CDF 1 mice (6 ~ 7 weeks old, body weights: 18 ~ 23 g) to which a carbon clearance test sample had been administered, and after 50 μL of a blood sample collected from ocular fundus veins had been mixed with 3 mL of a 0.1% sodium carbonate solution upon the passage of 1, 3, & 5 minutes since the dispensing, the microphage functions of livers & pancreas were analyzed by calculating the intake function coefficient (K value) based, as a parameter, on the disappearance of the carbon particles within blood at the time of the measurement of absorbance at 675 nm. Incidentally, a yeast extract derived from a commercial beer yeast and a commercial immunopotentiation agent (Inorich 50M, trademark of & manufactured by Eizai Co.) were concomitantly subjected to carbon clearance tests for comparative purposes. The obtained results are shown in Table V.

[0027]

[Table 5]

E xamination target	Administration dosage (mg/kg/day)	K value
Control	-	0.048 ± 0.013
Product 1 of the present invention	50	0.066 ± 0.002
Product 1 of the present invention	100	0.079 ± 0.008
Product 1 of the present invention	200	0.099 ± 0.003
Product 2 of the present invention	50	0.082 ± 0.003
Product 2 of the present invention	100	0.111 ± 0.017
Product 2 of the present invention	200	0.128 ± 0.002
Beer yeast extract	50	0.039 ± 0.001
Beer yeast extract	100	0.046 ± 0.007
Beer yeast extract	200	0.057 ± 0.001
Inorich 50M	50	0.051 ± 0.009
Inorich 50M	100	0.072 ± 0.009
Inorich 50M	200	0.095 ± 0.002

[0028]

(Effects of the invention)

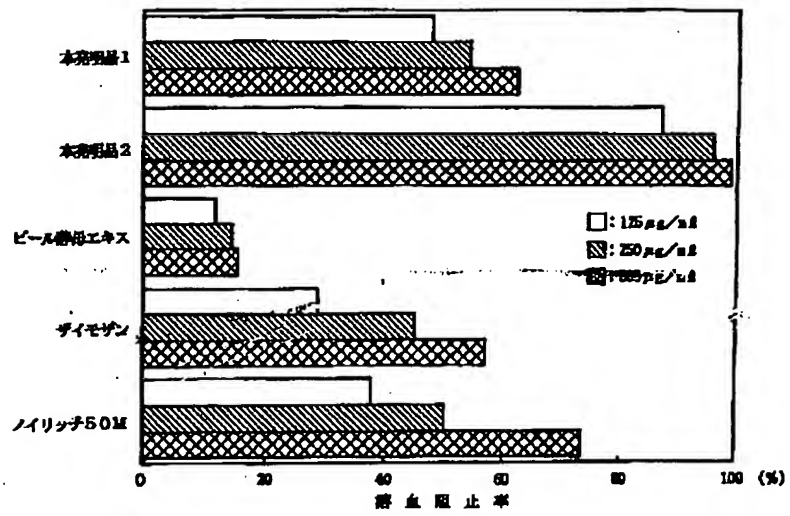
It becomes possible, according to the present invention, to obtain, both efficiently and inexpensively, a yeast extract composition endowed with both a fodder intake acceleration function and an immunopotentiation function unknown in the prior art. It is, furthermore, totally devoid of the fear of toxicity in that it is a natural product, and therefore, it can be safely added to a wide variety of fodders for preventing the breakouts of various infectious diseases.

Brief explanation of the figures /6

Figure 1: A diagram which shows, in terms of hemolysis blockage ratio (%), anti-complement activity test results on samples of the present invention, a beer yeast extract, Zymozan, and a commercial immunopotentiation agent (Inorich 50M).

Figure 2: A diagram which shows glucose consumption ratios (%) ascertained during microphage activation tests pertaining to cases where the samples of the present invention, a beer yeast extract, Zymozan, and a commercial immunopotentiation agent (Inorich 50M) were added and where none were added.

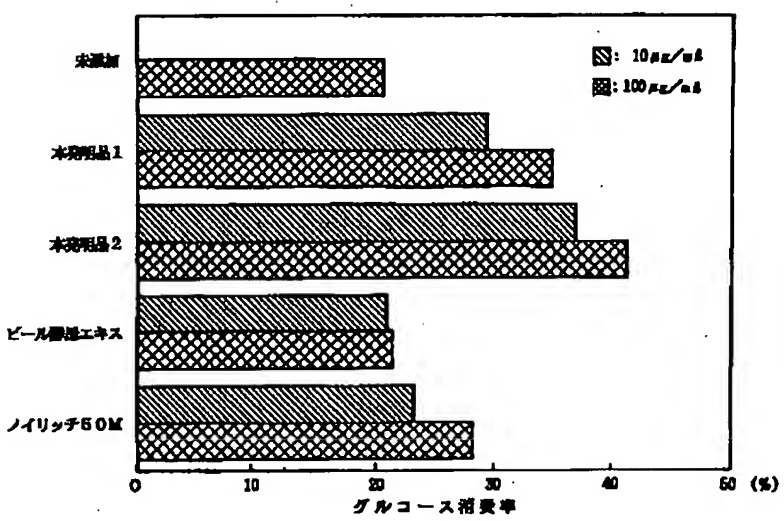
Figure 1



[(1): Sample 1 of the present invention; (2): Sample 2 of the present invention; (3): Beer yeast extract; (4): Zymozan; (5): Inorich 50M; (6): Hemolysis blockage ratio]

Figure 2

【図2】



[(1): None added; (2): Sample 1 of the present invention; (3): Sample 2 of the present invention; (4): Beer yeast extract; (5): Inorich 50M' (6): Glucose consumption ratio]

[Procedural amendment form]

[Date filed]: March 30, Hei 6[1994]

[Procedural Amendment 1]

[Name of document targeted for amendment]: Specification

[Name of item targeted for amendment]: 0003

[Amendment method]: Alteration

[Contents of amendment]

[0003]

As far as alternative methods are concerned, a method wherein a substance endowed with a fodder intake acceleration function is administered for purposes of accelerating growths and of abbreviating the duration of juvenile periods, which are prone to infectious diseases, a method wherein an immunopotentiation substance is administered for the purpose of inducing the acquisition of resistance against infectious diseases, etc. are being investigated. Known substances endowed with fodder intake acceleration functions are instantiated by 5'-nucleotide, free amino /7 acid, peptide, sugar, etc. A method wherein a substance inclusive of these substances as effective components is added to a fodder for purposes of improving the appetite and of accelerating the fodder intake is known (Japanese Patent Application Publication Kokai No. Hei 3[1991]-266944). There exists, however, a limit to the extent that the duration of juvenile periods prone to infectious diseases can be abbreviated, and thus, it cannot be said to be a fully satisfactory method by itself.

[Procedural Amendment 2]

[Name of document targeted for amendment]: Specification

[Name of item targeted for amendment]: 0014

[Amendment method]: Alteration

[Contents of amendment]

[0014]

Fodder intake acceleration functions & effects of four samples, namely the obtained two types of yeast extracts, a yeast extract derived from a commercial beer yeast, and a commercial grape juice added as a fodder intake accelerator at the suckling stage, were investigated.

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DERWENT-WEEK: 199826

TITLE: Yeast extract compsn for animal feed - contains 5'-nucleotide(s) and has immunostimulant activities

PATENT-ASSIGNEE: NIPPON SEISHI KK[NISEN]

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ABSTRACTED-PUB-NO: JP 07184595A

BASIC-ABSTRACT:

Yeast, partic. Torula or Saccharo yeast, esp. cultured in sulphite pulp waste liquor, extract compsn. having aperitive and immunostimulant activities and contg. 5'-nucleotides, partic. 1-5% each of 5'-inosinic acid and 5'-guanylic acid, free amino acids, partic. at concns. of 12-40%, beta-glucan, partic. at concns. of 1-25%, and mannan, partic. at concns. of 1-25%, prepd. by autolysis, partic. at 45-65 deg.C and pH 5.5-8.5, heating to inactivate intracellular enzymes, partic. at 80-120 deg.C, successively followed by action of cell wall degrading enzyme, 5'-phosphodiesterase and 5'-adenylic acid deaminase to enrich the essential components and used for feed, partic. at concns. of 0.1-20 wt.% in the feed.

Yeast including Torula and Saccharo spp are starting materials. Yeasts (e.g. Saccharomyces cerevisiae IFO 1954, 0309, IAM 4274, Candida utilis IFO 0619, Torulopsis sterata IFO 1953, Hansenula anomala IFO 1150) are cultured in sulphite pulp waste liquor and suspended at concns. of 10-15% to cause autolysis at 45-65 deg.C and pH 5.5-8.5 for 10-20 hrs. The autolysed mixt. is heated to 80-120, pref. 90-100 deg.C to inactivate the intracellular enzyme and

mixed with 0.3-3% of cell wall degrading enzyme (e.g. glucanase and mannase) and incubated for 1-5 hrs. The incubated mixt. is heated at 90 deg.C, centrifuged to give a supernatant and added to feeds at concns. of 0.1-20, pref. 0.2-5%.

USE/ADVANTAGE - Animal feed including fish and fowls. Feeds contg. 0.1-20 wt.% of the yeast extract. Improved aperitive and immunostimulant activities.

In an example, artificial milk for piglet was mixed with the supernatant or commercial feed additives at a concn. of 0.2% and gave 10 piglets for 14 days. The ratio of consumed feed for control gp., the supernatant, commercial yeast extract and grape juice was 100, 117-119, 101 and 103, respectively.

CHOSEN-DRAWING: Dwg.0/2

TITLE-TERMS: YEAST EXTRACT COMPOSITION ANIMAL FEED CONTAIN NUCLEOTIDE IMMUNOSTIMULANT ACTIVE

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(54)【発明の名称】　酵母エキス組成物及びその製造法並びにそれを含有する飼料

(57)【要約】

【目的】　従来の酵母エキスにはみられない摂餌促進作用並びに免疫増強作用を共に有する酵母エキス組成物を開発する。

【構成】　５’－ヌクレオチド、遊離アミノ酸、β－グルカン及びマンナンとを有効成分とする摂餌促進作用並びに免疫増強作用を有する酵母エキス組成物及びその製造法並びそれを含有する飼料。

【効果】　各種感染症の予防を目的として、広く飼料添加物として利用出来る。

1

【特許請求の範囲】

【請求項1】　5'－ヌクレオチド類、遊離アミノ酸，β－グルカン及びマンナンを含有することを特徴とする摂餌促進作用及び免疫増強作用を有する酵母エキス組成物。

【請求項2】　5'－イノシン酸、5'－グアニル酸を対固形分当り各々1～5%含有し、且つ遊離アミノ酸を対固形分当り12%～40%とβ－グルカンを1%～25%とを含有し、更にマンナンを1%～25%含有する請求項1記載の酵母エキス組成物。

【請求項3】　自己消化を行わせた後に加熱し、菌体内酵素を総べて失活後、細胞壁溶解酵素を作用させ、更に続いて5'－ホスホジエステラーゼ、5'－アデニル酸デアミナーゼを作用させて5'－ヌクレオチド含量・遊離アミノ酸含量、β－グルカン及びマンナン含量を共に高めることを特徴とする酵母エキス組成物の製造法。

【請求項4】　自己消化条件が温度45～65℃、pH5．5～8．5である請求項3記載の酵母エキス組成物の製造法。

【請求項5】　加熱時の温度が80～120℃である請求項3または4記載の酵母エキス組成物の製造法。

【請求項6】　酵母がトルラ酵母またはサッカロ酵母である請求項3～5中の何れか1項に記載の酵母エキス組成物の製造法。

【請求項7】　酵母が亜硫酸パルプ排液で培養した酵母である請求項3～6中の何れか1項に記載の酵母エキス組成物の製造法。

【請求項8】　請求項1～2中の何れか1項に記載の酵母エキス組成物を含有する飼料。

【請求項9】　請求項1～2中の何れか1項に記載の酵母エキス組成物を0．1～20重量%含有する飼料。

【発明の詳細な説明】

【0001】

【産業上の利用分野】本発明は酵母エキス組成物及びその製造法並びにそれを含有する飼料に関し、詳しくは摂餌促進作用及び免疫増強作用を共に有する酵母エキス組成物及びその製造法並びにそれを含有する飼料に関するものである。

【0002】

【従来の技術及びその問題点】魚類、家畜及び家禽類の幼若期は一般に免疫機能が充分でなく、そのため消化管系・呼吸器系の感染症が発生し易い。よって出来るだけ速く増体させ抵抗力を付けることが必要である。また一度この様な感染症が発生すると、一般的に魚類、家畜及び家禽類の飼育は、生産効率向上のため高密度で行われているために蔓延し易く、経済的損失は極めて大きく、特に水畜産業界に於いては重要な問題点となっている。現在これ等の感染症の予防・治療には、抗生物質を始めとした種々の薬剤が使用されている。しかしながらこれ等薬剤の効果は充分でない上に、新たに薬剤の体内残留

2

・薬剤耐性菌の出現といった問題が生じ、薬剤の使用は制限される方向にある。

【0003】これ等に代わる方法として、摂餌促進作用を有する物質を投与して増体を促し、感染症に掛かり易い幼若期の期間を短くする方法、免疫増強物質を投与して感染症に対する抵抗力を付ける方法などが検討されている。摂餌促進作用を有する物質としては、5'－ヌクレオチド類、遊離アミノ酸、ペプチド、砂糖等を知られている。これ等を有効成分とする物質を飼料に添加して嗜好性を改善し摂餌を促進させる方法が知られている（特開平3－266944）。しかしながら感染症に掛かり易い幼若期の期間を短縮するには限度があり、これだけでは充分満足の行く方法とは言えなかった。

【0004】また免疫増強物質としてはキノコや酵母菌等の細胞壁構成成分であるβ－グルカンやマンナン等が知られている。キノコ由来の免疫増強物質としては、シイタケから熱水抽出されたレンチナンやスエヒロタケが生産するシゾフィラン等が開発上市されている。また酵母菌体を用いた免疫増強剤としては、酵母菌体の細胞壁構成成分であるβ－グルカンを含むザイモザンが知られている。しかしながらこれ等の免疫増強剤は、製造工程が煩雑で収量も充分でないためにコスト高となり、飼料添加物として広く利用するには問題があった。また免疫増強作用と体重増加作用を共に有する物質に就いても知られているが（特開平2－11519）、飽くまで強制的に摂取させた時にのみ有効であり、その効果に就いても充分満足の行くものではなかった。以上の様な理由で、摂餌促進作用が有り、且つ免疫増強作用も有する物質の開発が熱望され続けていた。

【0005】

【課題を解決するための手段】本発明者等は上記課題を解決すべく鋭意研究した結果、温度・pHを特定の範囲に限定した自己消化を行い、固形分収率を上げ、且つ高分子RNA及びマンナンを分解させる事なく多量の遊離アミノ酸含量を増加させた後に、反応液を一定条件で加熱し菌体内酵素を失活させ、次ぎに細胞壁溶解酵素を作用させ、更に続けて5'－ヌクレオチドを生成する5'－ホスホジエステラーゼ及び5'－アデニル酸デアミナーゼを添加すると、摂餌促進作用並びに免疫増強作用が共に非常に優れた酵母エキス組成物が得られることを発見し、本発明を完成するに至った。

【0006】本発明に於ける自己消化の温度・pHは、遊離アミノ酸含量を高めること並びにRNA及びマンナンの分解を抑える点で、また自己消化反応後に加熱工程を組み入れることは、以後の酸素反応工程に於いて呈味性5'－ヌクレオチド及びβ－グルカン・マンナンの分解を抑制する点で極めて重要な因子となるものである。以下に本発明を更に詳細に説明する。本発明で使用する酵母は、食用または飼料用のものであれば特に制限は無く、ビール酵母，パン酵母，アルコール酵母，清酒用酵

3

母など一般に食品工業で用いられているものを使用することが出来る。

【0007】このような酵母の例としては、サッカロマイセス・セレビシェ（IFO 1954，IFO 0309，IAM 4274）、キャンデイダ・ユーティリス（IFO 0619，ATCC 15239）、トルロプシス・ノダエンシス（IFO 1942）、トルロプシス・ステラタ（IFO 1953）、ハンセヌラ・アノマラ（IFO 1150）等が挙げられる。中でもトルラ酵母はRNA含量が高く、呈味力も強いので好ましい。酵母菌体は培養後、洗浄して得られる生菌体を使用するが、特に亜硫酸パルプ排液で培養した酵母が、安価な上に活性が高いので本発明の酵母として優れている。

【0008】酵母を10～15%程度の適当な濃度に懸濁させた後、自己消化反応を行う。反応pH及び反応温度に就いては、高分子RNA及びマンナンの分解を抑えると共に遊離アミノ酸生成を高める様な条件が必要であり、pH5．5～8．5、温度45～65℃の範囲に於いて目的は達成される。pHに就いてはこの範囲以外に於いては遊離アミノ酸含量を上げることが困難である。温度に就いてはこの範囲より下では遊離アミノ酸含量は高くなる反面、RNA及びマンナンの分解がみられる。また65℃を超えるとRNA及びマンナンの分解は無くなるが、遊離アミノ酸含量は極端に低下して了う。遊離アミノ酸含量が12%未満では著明な摂餌促進効果が認められない。また40%を超える含量とするには自己消化時間が極めて長くなり、腐敗等の問題が生じて来る。よって遊離アミノ酸含量としては12%～40%が好ましい。

【0009】上記の条件下で自己消化を10～20時間程度行わせた後、80～120℃好ましくは90～100℃で加熱し、菌体内酵素の失活を行う。加熱時間は10分程度で充分である。次ぎに細胞壁溶解酵素を0．3～3%程度添加して、1～5時間反応させる。この範囲の時間内に於いては、先の工程を経て来た酵母は細胞壁溶解酵素を添加しても、あまり多糖類の低分子化を伴わずに固形分収率を上げることが出来る。これは細胞壁中のグルカン・マンナンは蛋白質との複合体を形成しているが、加熱処理により蛋白質が変性して細胞壁構造が堅固になり、低分子化が阻害を受けるものと考えられる。反応時間が短いとβ－グルカン，マンナン含量が1%未満となり、免疫活性が充分でない上に固形分収率が低下して了う。これ以上長過ぎるとβ－グルカン，マンナン含量は25%以上出せるが、必要以上に低分子化して了い、免疫活性が低下して了う。よってβ－グルカン，マンナン含量は共に1～25%が好ましい。

【0010】使用する細胞壁溶解酵素剤としてはグルカナーゼ，マンナナーゼを含有し、酵母細胞壁を溶解するに充分な活性を有するものであれば構わないが、例えば

4

市販の細胞壁溶解酵素としては、YL－5（天野製薬（株）製），ツニカーゼ（大和化成（株）製，キタラーゼ（クミアイ化学（株）製）などが挙げられる。引続き5'－ホスホジエステラーゼ，5'－アデニル酸デアミナーゼを添加し、5'－ヌクレオチド類を生成させる。酵素添加量，酵素反応温度，pHは特に限定するものではなく、各々の酵素の最適条件下で行えばよい。反応終了後、反応液は90℃に加熱し酵素を失活させた後、遠心分離して上澄液を濃縮しエキス分として回収し、スプレードライ等の方法により乾燥させる。この様にして得られた酵母エキスは、5'－イノシン酸・5'－グアニル酸を共に対固形分当り1～5%、遊離アミノ酸を12～40%含有しているため、強い摂餌促進効果を有している。なお且つβ－グルカンを1～25%、マンナンを1～25%含有しているため、優れた免疫増強活性を有している。また固形分収率も50%以上あるため経済的にも非常に有利であり、広く飼料添加物として利用出来る。本発明品の飼料への添加剤は対象とする動物の種類、週齢により異なってくるが、0．1～20重量%、好ましくは0．2～5%の範囲で添加すれば本発明は達成できる。

【0011】

【実施例】以下に具体的な実施例を示すが、本発明はこれに限定されるものではない。

【0012】摂餌促進活性の測定

試作例1

サッカロマイセス・セレビシェ（IFO 1954）を5%糖蜜培地を用いて培養し、集菌洗浄後酵母スラリー（菌体濃度15%）1000mlを調製した。pHを6に調製した後、55℃にて18時間反応させた。反応後、90℃、10分間加熱し菌体内酵素を失活させた後に、細胞壁溶解酵素（商品名：YL－5（天野製薬（株）製））を1．5g添加し55℃にて3時間反応させた。次ぎに70℃まで加温し、5'－ホスホジエステラーゼ（商品名：ヌクレアーゼ「アマノ」（天野製薬（株）製））を0．3g添加しpH5に調製後、10時間反応させた。続いて5'－アデニル酸デアミナーゼ（商品名：デアミザイム（天野製薬（株）製））を0．2g添加しpH5に調製後、10時間反応させた。反応後、常法により処理し122gの酵母エキスを得た。この酵母エキス中の5'－イノシン酸、5'－グアニル酸、遊離アミノ酸含量を高速液体クロマトグラフを用いて定量したところ、含量は各々2．5%、2．6%、45%であり固形分収率は81．3%であった。またβ－グルカン及びマンナンの含量を試料を高速液体クロマトグラフを用いて加水分解前後の差から定量したところ、含量はそれぞれ9%、8%であった。更にこれ等の分子量をゲル沪過法で求めたところ、各々6．3万、5．9万であった。

【0013】試作例2

5

トルラ酵母を３％亜硫酸パルプ排液培地を用いて培養し、集菌洗浄後、酵母スラリー（菌体濃度１５％）１０００ｍｌを調製した。ｐＨを６．５に調製した後、６０℃で１８時間自己消化反応を行った。その後９５℃、１０分間加熱し菌体内酵素を失活させた後、細胞壁溶解酵素（商品名：ツニカーゼ（大和化成（株）製））を１．８ｇ添加し５５℃にて２．５時間反応させた。反応後、７０℃まで加温し核酸分解酵素（商品名：ヌクレアーゼ「アマノ」（天野製薬（株）製））を１８０ｍｇ添加し９時間反応させた。その後、４５℃まで温度を下げプロテアーゼ（商品名：アマノＰ（天野製薬（株）製））１．８ｇ、５’－アデニル酸デアミナーゼ（商品名：デアミザイム（天野製薬（株）製））２００ｍｇを添加し１０時間反応させた。冷却後、常法により処理し１０５ｇの酵母エキスを得た。この酵母エキス中の５’－イノシン酸、５’－グアニル酸、遊離アミノ酸含量を高速液体クロマトグラフを用いて定量したところ、含量は各々３．６％、３．８％、３５％であり固形分収率は７０．０％であった。またβ－グルカン及びマンナンの含量を高速液体クロマトグラフを用いて定量したところ、含量はそれぞれ１２％、２０％であった。更にこれ等の分子量をゲル沪過法で求めたところ、各々７．２万、５．６万であった。

【００１４】参考

シゾフィラン	MW	１万～８万
レンチナン		４０万
パヒマラン		１８万
ザイモザン中のグルカン		６５００
酵母分解中のマンナン		５．９万 ＊

6

＊他社酵母エキス中の推定マンナン含量　３％以下
他社酵母エキス中の推定グルカン含量　１％以下
ＨＵ中のマンナン含量　１２％　MW　７．２万
得られた２種類の酵母エキスと市販のビール酵母発底の酵母エキス、並びに哺乳期に摂餌促進剤として添加される市販のぶどう果汁の４つのものに於いて、摂餌促進作用効果を調べた。

【００１５】実施例１

子豚に於いては表１に示した基本飼料を基に、各種試料を０．２％添加して飼料を調製した。

【００１６】

【表１】表１　子豚用人工乳の基本飼料組成

〔成分〕	〔配合量（重量％）〕
小麦粉	３５
脱脂粉乳	３５．５
大豆蛋白	１０
魚粉	４
ブドウ糖	１０
油脂	３
ビタミン・ミネラル	２
乳化剤	０．５

【００１７】同腹離乳豚（２週齢）１０頭をそれぞれ飼育ゲージに収容し、対照と各試料が添加された飼料とを１４日間選択法により摂食させ、摂食量を比較した。なお供試飼料箱は１日交代で置き場を交互に変えた。結果は表２の通りである。（摂食量比：対照の人工乳の摂食量を１００に換算した値を示す（以下同じ））。

【００１８】

【表２】

表２　各試料の摂食量比

試料名	対照	本発明品１	本発明品２	市販酵母エキス	ぶどう果汁
摂食量比	１００	１１７	１１９	１０１	１０３

【００１９】実施例２

子牛に於いては基本飼料として市販子牛用人工乳を用い、各種試料を０．２％添加した飼料を調製した。次ぎに母畜から離された１週齢の幼牛を各群６頭宛用い、実※施例１と同様な方法で評価した。結果は表３に示す通りであった。

【００２０】

【表３】

表３　各試料の摂食量比

試料名	対照	本発明品１	本発明品２	市販酵母エキス	ぶどう果汁
摂食量比	１００	１１６	１２０	１０１	１０３

【００２１】実施例３

雛に於いては基本飼料として市販幼雛期用飼料を用い、各種試料を０．２％添加した飼料を調製した。次ぎに１週齢の幼雛を各群１０羽宛用い、実施例１と同様な方法★で評価した。結果は表４に示す通りであった。

【００２２】

【表４】

表4　各試料の摂食量比

試料名	対照	本発明品1	本発明品2	市販酵母エキス	ぶどう果汁
摂食量比	100	115	118	102	104

【0023】免疫増強活性の測定
試作例1，2で作った飼料を用いて以下の試験を行った。

【0024】実施例1　抗補体活性試験
適度に希釈したモルモット血清から成る補体溶液に試料を添加し、37℃にて30分間保温した。次いで、抗体で感作した羊赤血球を加え、37℃にて60分間保温した後、羊赤血球の溶血度を測定することにより、試料よって活性化されなかった残存補体量を測定し、試料の補体第二経路活性化作用を測定した。なお、比較のために市販のビール酵母発底の酵母エキス、ザイモザン（Sigma株式会社製：酵母細胞壁成分の商品名）及び市販免疫増強剤（エーザイ株式会社製：商品名ノイリッチ50M）に就いて同時に抗補体活性試験を行った。その結果を図1に示す。

【0025】実施例2　マクロファージ活性化試験
チオグリコレート培地で誘導したマウス腹腔内浸出細胞に各試料を添加し、24時間後の培養上清中のグルコース量を定量し、その消費量からマクロファージに対する活性化作用を測定した。なお、比較のために市販のビール酵母発底の酵母エキス及び市販免疫増強剤（エーザイ株式会社製：商品名ノイリッチ50M）に就いても同時にマクロファージ活性化試験を行った。その結果を図2に示す。

【0026】実施例3　カーボンクリアランステスト
試料を投与したCDF1マウス（雌6～7週齢、体重18～23g）の尾静脈中に、25倍に希釈したカーボン粒子（ロットリングインキで代用）を注入し、注入後1，3及び5分経過した後に、眼底静脈より採取した50μlの血液を3mlの0．1%炭酸ナトリウム溶液と混合し、675nmの吸光度を測定したときのカーボン粒子の血中消失を指標として食作用係数（K値）を算出することにより、肝臓と脾臓のマクロファージ機能の測定を行った。なお、比較のために市販のビール酵母発底の酵母エキス並びに市販免疫増強剤（エーザイ株式会社製：商品名ノイリッチ50M）に就いても同時にカーボンクリアランステストを行った。その結果を表5に示す。

【0027】
【表5】

検体	投与量(mg／kg／日)	K値
コントロール	－	0．048±0．013
本発明品1	50	0．066±0．002
本発明品1	100	0．079±0．008
本発明品1	200	0．099±0．003
本発明品2	50	0．082±0．003
本発明品2	100	0．111±0．017
本発明品2	200	0．128±0．002
ビール酵母エキス	50	0．039±0．001
ビール酵母エキス	100	0．046±0．007
ビール酵母エキス	200	0．057±0．001
ノイリッチ50M	50	0．051±0．009
ノイリッチ50M	100	0．072±0．009
ノイリッチ50M	200	0．095±0．002

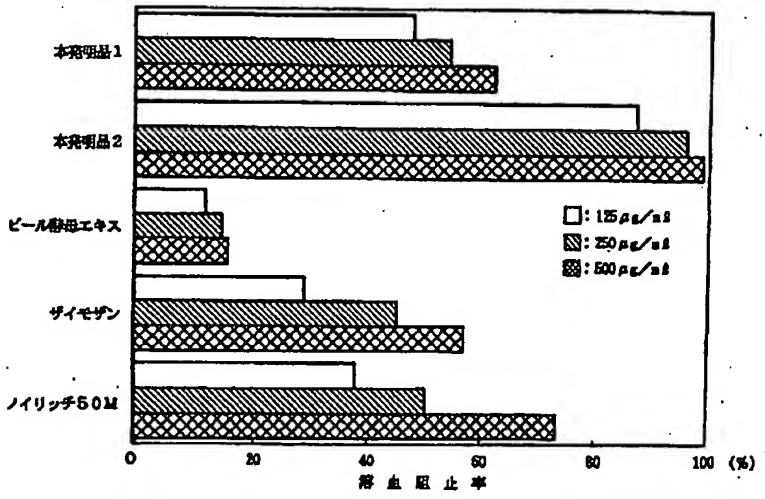
【0028】
【発明の効果】本発明によれば、従来に存在してなかった摂餌促進作用及び免疫増強作用を有する酵母エキスを※効率良く、しかも安価に得ることが出来る。またこのものは天然物であるために毒性も全く無く、安心して各種感染症の発生予防に広く飼料に添加することが出来る。

【図面の簡単な説明】

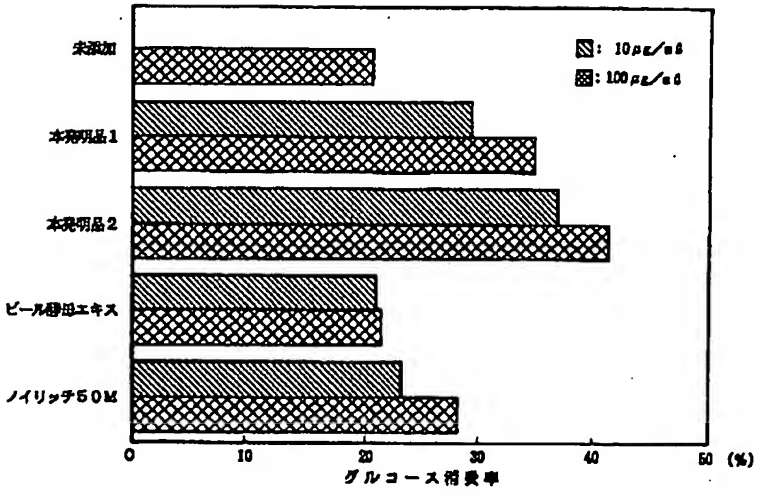
【図1】本発明品とビール酵母エキス，ザイモザン，市販免疫増強剤（ノイリッチ５０Ｍ）に就いての抗補体活性試験結果を溶血阻止率（％）によって示した図である。

【図2】本発明品と市販のビール酵母エキス，市販免疫増強剤（ノイリッチ５０Ｍ），及び未添加の場合とに就いてマクロファージ活性化試験を行ないグルコース消費率（％）で示した図である。

【図1】



【図2】



【手続補正書】
【提出日】平成６年３月３０日
【手続補正１】
【補正対象書類名】明細書
【補正対象項目名】０００３
【補正方法】変更

【補正内容】
【０００３】これ等に代わる方法として、摂餌促進作用を有する物質を投与して増体を促し、感染症に掛かり易い幼若期の期間を短くする方法、免疫増強物質を投与して感染症に対する抵抗力を付ける方法などが検討されて

いる。摂餌促進作用を有する物質としては、5’－ヌクレオチド類、遊離アミノ酸、ペプチド、砂糖等が知られている。これ等を有効成分とする物質を飼料に添加して嗜好性を改善し摂餌を促進させる方法が知られている（特開平3－266944）。しかしながら感染症に掛かり易い幼若期の期間を短縮するには限度があり、これだけでは充分満足の行く方法とは言えなかった。

【手続補正2】

【補正対象書類名】明細書
【補正対象項目名】0014
【補正方法】変更
【補正内容】
【0014】得られた2種類の酵母エキスと市販のビール酵母発底の酵母エキス、並びに哺乳期に摂餌促進剤として添加される市販のぶどう果汁の4つのものに於いて、摂餌促進作用効果を調べた。